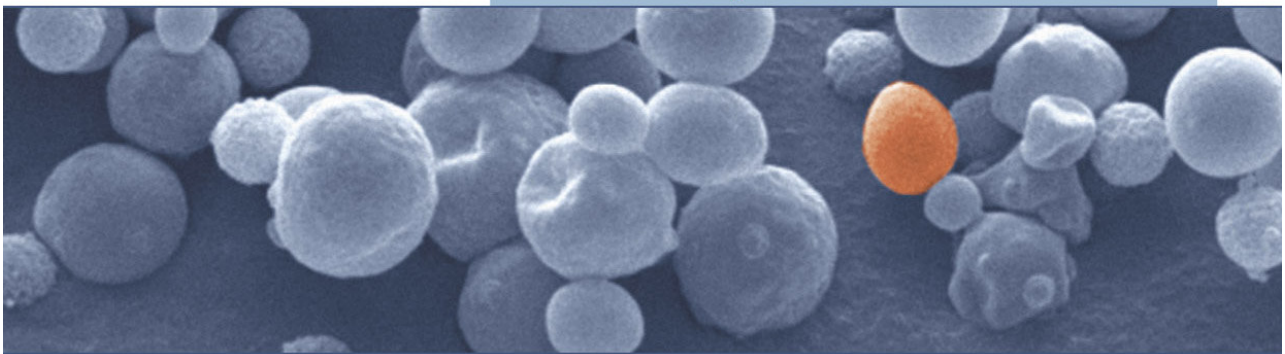


Dualsystems Biotech



KickStart™

Protein Expression Kit

P01006

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Thank you for purchasing the KickStart™ Protein Expression Kit !

KickStart™ protein expression kit

The KickStart™ protein expression kit has been specifically designed for inducible, high-level expression of recombinant proteins in *Saccharomyces cerevisiae*.

KickStart™ expression vectors combine two important features for protein expression:

- **Inducible *ADH2* promoter:** The *ADH2* promoter is induced when glucose becomes depleted in the medium. During early growth in YPD medium glucose levels are high and the *ADH2* promoter is inactive - thereby preventing negative effects on yeast growth. Once the cells reach the early stationary phase glucose is depleted from the medium and the *ADH2* promoter is induced, producing high amounts of protein.
- **KanMX cassette:** KickStart™ vectors feature a KanMX cassette, which allows selection with G418 in rich medium. The use of rich medium such as YPD will significantly enhance cell densities and protein yield.

Support

Should you encounter any problems during the application of the KickStart™ system, please consult our support pages at www.dualsystems.com. Support protocols and our **Knowledge Base** are constantly updated and hold answers for most commonly encountered problems when working with yeast. If you cannot find answers to your questions in our support pages, contact us at support@dualsystems.com and we will help you as quickly as possible.

Newsletter

We also offer a Newsletter with tips & tricks for working with yeast, discussions of recent literature and descriptions of novel products. Please go to www.dualsystems.com to subscribe.

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1 Kit contents

The KickStart™ protein expression kit contains the following components:

Component	Supplied as
pKS1 Expression vector	5 µg lyophilized plasmid
pKS1-ST Strep-tag expression vector	5 µg lyophilized plasmid
pKS1-ST-Gal Control vector	5 µg lyophilized plasmid
DSY-5 Yeast expression strain	MATalpha leu2 trp1 ura3-52 his3::GAL1-GAL4 pep4 prb1-1122 Lyophilized yeast culture
XT Extraction buffer	1 x 50 ml
Glass beads	1 x 20 ml

Storage and handling of plasmids

Plasmids are supplied lyophilized. Upon receipt, add 50 µl water (final concentration 0.1 µg/µl), vortex for 1 minute, incubate at 50°C for 5 minutes and vortex again for 1 minute. Use 0.5-1 µl for transformation of competent *E. coli* cells. Store remaining plasmid at -20°C.

Storage and handling of yeast strains

To rehydrate the strain, add 50 µl sterile Millipore quality water and let stand for 10 minutes at room temperature. Resuspend by gentle shaking (some undissolved clumps may remain, this does not decrease the performance of the strain) and streak out 5 µl and 40 µl on fresh YPAD plates. Incubate at 30°C for 2-3 days, you should see 1-2 mm diameter white/pinkish colonies. Store plate at 4°C, restreak every week.

Preparing glycerol stocks

Inoculate several colonies into 20 ml liquid YPD medium and grow overnight at 30°C with shaking. Pellet cells by centrifugation at 2500 g for 5 minutes, discard the supernatant and resuspend the pellet in 10 ml freezing mix (YPD medium supplemented with 25 % glycerol). Aliquot into cryotubes and place at -80°C (do NOT flash freeze in liquid nitrogen!). Glycerol stocks are viable for several years when stored at -80°C.

Restreaking glycerol stocks

To restreak a stock, remove a cryotube from the freezer and scratch off the yeast from the surface of the frozen stock using a sterile loop. Streak onto a YPD plate, seal the plate with parafilm and incubate at 30°C for 2-3 days. Yeast plates can be stored at 4°C for 1 month. Do not use yeast older than 2 weeks for transformation.

2 Introduction

Recombinant protein expression in yeast

Bakers yeast, *Saccharomyces cerevisiae*, is one of the most intensively investigated eukaryotic organisms and has been widely used as a host for recombinant protein expression [1-3].

Saccharomyces cerevisiae has several properties which make it a particularly attractive host for foreign protein expression:

- It is classified as a GRAS (generally regarded as safe) organism
- The yeast cell wall does not contain any toxic components (such as pyrogens in the case of *E. coli*)
- It is easy to cultivate and no expensive equipment or media are needed
- Using appropriate vector systems, high amounts of proteins can be produced
- Recombinant proteins are generally soluble and retain their native fold; insolubility problems are rarer than in bacterial host such as *E. coli*
- Recombinant proteins can be secreted into the medium
- Posttranslational modifications are retained

Yeast expression vectors

Most general yeast expression vectors contain a common set of elements:

- An origin of replication derived from the yeast endogenous 2 μ circle. This origin of replication governs stable plasmid propagation at approximately 20-100 copies per cell.
- A selection marker for propagation in yeast, normally an auxotrophic marker such as *TRP1*, *LEU2*, *URA3* or *HIS3*.
- Elements for selection and propagation of the plasmid in *E. coli*
- A promoter which is active in yeast
- A multiple cloning site for cDNA insertion
- An appropriate terminator sequence

Inducible promoters

Constitutively active promoters, such as the alcohol dehydrogenase (*ADH1*) or translation elongation factor 1 (*TEF1*) promoter have the disadvantage that constitutive, high-level expression of slightly toxic proteins normally has a detrimental effect on cell growth and final cell density. For this reason, inducible promoters are preferred when expressing recombinant proteins in yeast.

The most commonly used inducible yeast promoter is the galactokinase (*GAL1*) promoter. Induction is carried out by exchanging glucose for galactose as carbon source. Although the *GAL1* promoter produces high-level expression and shows very good induction kinetics, the need to exchange the medium is very laborious, particularly when performing large-scale expressions in bioreactors [4].

The *ADH2* promoter

A less widely used inducible promoter is derived from the *Saccharomyces cerevisiae ADH2* gene encoding alcohol dehydrogenase II. In contrast to the *GAL1* promoter the *ADH2* promoter is not induced by addition of a particular compound

but instead, is repressed by glucose. Upon depletion of glucose the *ADH2* promoter is activated.

When yeast is grown with glucose as carbon source, the high glucose concentration during the initial (early exponential) growth phase prevents induction of the *ADH2* promoter. Once the cells reach the late exponential/early stationary phase, the *ADH2* promoter is activated and recombinant protein is expressed. Thus, the *ADH2* promoter combines two important advantages of an inducible promoter:

- Induction of protein expression is separated from the growth phase
- Induction does not require addition of a compound or exchange of growth medium

The *ADH2* promoter has been successfully used for protein expression, including expression of human cytokines [2], *Penicillium patulum* fungal polyketide synthase 6-methylsalicylic acid synthase [3] or *Closterium* sex pheromone [5].

The derepression mechanism of the *ADH2* promoter has been studied in depth: two cis-acting elements, UAS1 (upstream activation site) and UAS2/CSRE (carbon-source responsive element), are both necessary for maximal activation of *ADH2* [6,7]. In the absence of a fermentable carbon source, these sites are bound cooperatively by the transcriptional activators Adr1p and Cat8p [6,8,9]. The presence of glucose downregulates the levels of Adr1p and Cat8p, which in turn leads to the *ADH2* promoter being repressed several hundred-fold [6,10,11].

The KanMX marker

Many commonly available yeast expression vectors use auxotrophic markers such as *TRP1* or *LEU2* for selection. The use of auxotrophic markers requires a defined minimal medium (for example SD medium, see www.dualsystems.com for recipes). Defined minimal medium has several disadvantages, including low cell densities and hence decreased protein yields.

The use of rich medium (such as YPD) favours rapid growth and high cell densities and generally results in higher protein yields than minimal medium but unfortunately, auxotrophic markers cannot be used for plasmid selection in rich medium. Dominant resistance markers, on the other hand, can be used together with rich medium and offer an attractive means of selecting for the presence of a yeast expression plasmid.

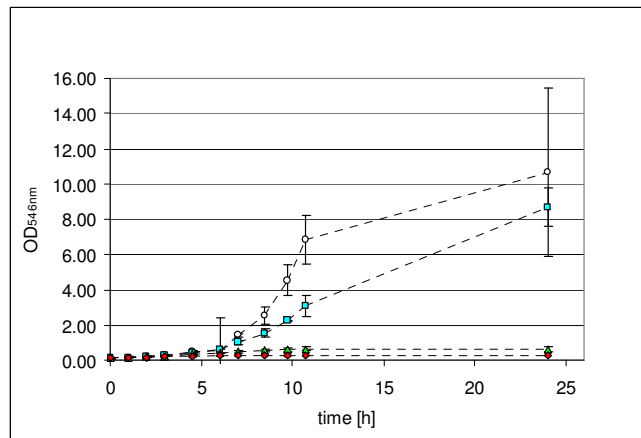
One of the most commonly used dominant selection markers in yeast is the so-called KanMX cassette, which contains the coding sequence of the *kan^r* gene of transposon *Tn903* encoding aminoglycoside phosphotransferase. Plasmids carrying the KanMX cassette can be selected using rich medium supplemented with the compound G418.

Selection using G418

Yeast strains vary in their sensitivity towards G418. In addition, the presence of large plasmids or plasmids encoding a toxic protein may also alter the G418 sensitivity of the yeast strain. For this reason, we always recommend to test the growth characteristics of your particular strain using several concentrations of G418.

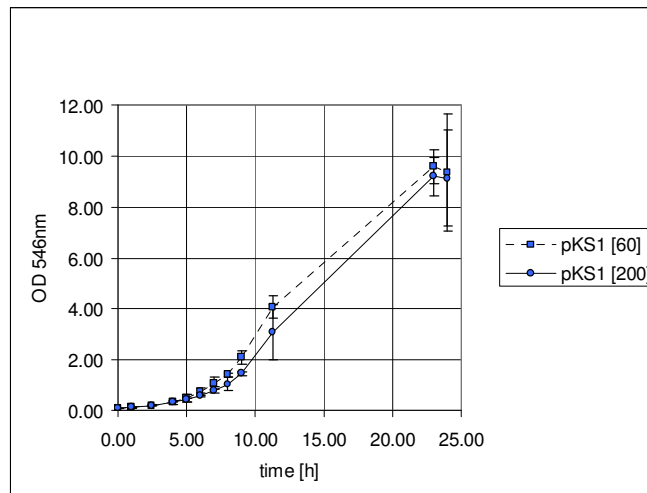
Below, a growth curve of DSY-5 at different G418 concentrations is shown. An equal amount of yeast was inoculated into YPD cultures supplemented with no

G418 (open circles), 20 $\mu\text{g/ml}$ G418 (cyan squares), 60 $\mu\text{g/ml}$ G418 (green triangles) or 200 $\mu\text{g/ml}$ G418 (red diamonds). At the indicated time points, 1 ml aliquots of the cultures were taken for measurement of $\text{OD}_{546\text{nm}}$. All measurements were done in triplicate and results from two separate growth experiments were pooled.



The growth experiment shows that 60 $\mu\text{g/ml}$ G418 in the medium is sufficient to inhibit growth of nontransformed DSY-5.

In a second experiment, DSY-5 transformed with pKS1 was used to establish a growth curve in YPD medium supplemented with 60 $\mu\text{g/ml}$ G418 (squares) or 200 $\mu\text{g/ml}$ G418 (circles).



DSY-5 transformed with pKS1 can be selected efficiently using both 60 and 200 $\mu\text{g/ml}$ G418.

pKS expression vectors

KickStart™ pKS expression vectors feature the *ADH2* promoter for easy induction and high-level production of recombinant proteins in yeast and the KanMX marker for plasmid maintenance and protein expression in rich medium. In addition, some vectors contain the popular Strep-tag™ sequence, which facilitates quick and easy protein purification using IBA's Strep-Tactin™ resin.

The following vectors are part of the KickStart™ protein expression kit:

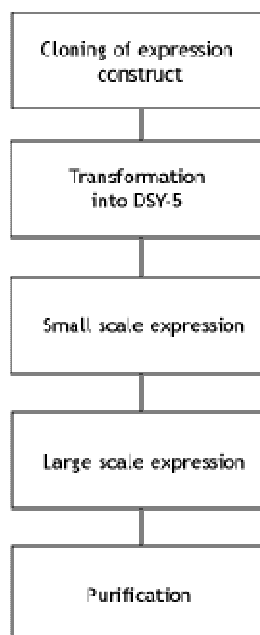
P03305	pKS1	Expression vector
P03306	pKS1-ST	Expression vector incorporating an N-terminal Strep-tag followed by an HA epitope tag

The following vectors are available separately:

P03308	pKS2-ST	Expression vector incorporating a <i>SUC2</i> signal sequence for secreted expression, an N-terminal Strep-tag and an HA epitope tag
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Expressing proteins in the KickStart™ system

The following section gives a brief overview of the different steps that need to be carried out for expression of recombinant proteins in yeast. A cDNA encoding the protein to be expressed is first cloned into any of the KickStart™ vectors. The resulting construct is transformed into the expression strain DSY-5 and expression of the protein of interest is tested in small scale cultures. Finally, a large scale expression culture is prepared and recombinant protein is purified using either specially adapted protocols or generic purification schemes, such as those using the Strep-Tag present in the vector pKS1-ST.



3 Protocols

Protocol	Content
1	Cloning of expression construct
2	Yeast transformation
3	Small scale expression
4	Large scale expression

Reagents

Reagents are listed in the Appendix.

Unless noted otherwise, use Nanopure water or water of similar quality.

Troubleshooting

If you have difficulties with a particular protocol or if you have a general question relating to yeast, please consult our Knowledge Base at www.dualsystems.com. If you cannot find an answer there, please contact us by email (support@dualsystems.com) and we will try to help as quickly as possible.

Protocol 1 Cloning of expression construct

Clone the cDNA encoding your protein to be expressed into a pKS vector using any of the restriction sites available in the multiple cloning site. When using pKS1-ST make sure that your cDNA is in frame with the upstream Strep-HA tag. Please consult the Appendix for pKS vector maps.

After you have cloned your cDNA into the pKS vector, verify your expression construct by sequencing. The following primers can be used to sequence cDNAs cloned into pKS vectors from the 5' and 3' ends.

pKS sequencing primers	
Primer	Sequence (5' > 3')
KS-U 5' sequencing primer Located in the <i>ADH2</i> promoter sequence	ATCAAGCTACAAAAAGCATAC
KS-R 3' sequencing primer Located in the <i>CYC1</i> terminator sequence	AAGCGTGACATAACTAATTAC

Protocol 2 Yeast transformation

Transform the expression construct(s) into DSY-5 as described in the protocol below:

Protocol



1. Inoculate 50 ml YPD with several colonies of DSY-5 taken from a fresh plate and grow overnight at 30 °C with shaking.

When transforming plasmids into yeast, always make sure that you are using colonies from a plate that is no older than 2 weeks. It is good practice to prepare several glycerol stocks from the original strain and use these for re-streaking at regular intervals. Always using fresh yeast ensures maximum transformation efficiencies and avoids unwanted artifacts.

2. Measure the OD₅₄₆ of the culture, which should be 0.6-0.8. If the OD₅₄₆ reading is above 1.0, dilute the culture to OD₅₄₆ of 0.2 and regrow to OD₅₄₆ 0.6.
3. Pellet the 50 ml culture for 5 minutes at 2500 g and resuspend in 2.5 ml water.
4. Prepare the PEG/LiOAc master mix (sufficient for 3 transformations):

PEG/LiOAc master mix	
Component	Amount
50 % PEG	720 µl
1 M LiOAc	108 µl
Single-stranded carrier DNA	75 µl



Please consult the Appendix for instructions on how to prepare 50 % PEG, 1 M LiOAc and single-stranded carrier DNA.

5. Set up the following reactions:

Reaction	Amount	Plasmid
1	1.5 µg	Expression construct
2	1.5 µg	pKS1-ST-Gal

6. Add 300 µl PEG/LiOAc master mix to each tube, vortex briefly.
7. Add 100 µl resuspended yeast cells from step 3 to each tube, vortex 1 minute to thoroughly mix all components.
8. Incubate in a 42 °C water bath for 45 minutes.
9. Pellet the cells for 5 minutes at 700 g.
10. Dissolve each pellet in 500 µl YPD and incubate at 30 °C for 90 minutes.
11. Pellet the cells for 5 minutes at 700 g.
12. Dissolve each pellet in 100 µl 0.9 % NaCl and plate each transformation onto the following plates:

Reaction	Plasmid	YPD (200 µg/ml G418)
1	Expression construct	100 µl
2	pKS1-ST-Gal	100 µl

- Seal all plates with parafilm and incubate for 3 days at 30°C.

Note

Selection on YPD (200 µg/ml G418) plates may result in spurious growth of colonies which do not contain a plasmid. Thus, your plates may contain a mix of true transformants (usually colonies with a diameter > 2 mm) and background colonies (usually colonies with a diameter < 2 mm). To separate true transformants from background colonies are picked from the primary plates and re-streaked onto fresh YPD-kan plates. Only true transformants show robust growth after 2 days incubation at 30°C. To increase the stringency of selection, the G418 concentration may be raised up to 400 µg/ml.

- Pick 10-20 single colonies (diameter > 2 mm) and re-streak onto fresh YPD (200 µg/ml G418) plates.
- Incubate for 2-3 days at 30°C. This is your master plate.

Results



True transformants show robust growth when re-streaked on YPD (200 µg/ml G418) plates. Pick several true transformants and prepare glycerol stocks for later use.

If you observe no colonies at all on the primary YPD (200 µg/ml G418) plates, your transformation most likely had a very low efficiency rate. Reasons for a low transformation efficiency may include:

- Bad quality single stranded DNA. Run 500 ng of single stranded carrier DNA on a 1 % agarose gel. You should see a smear ranging from > 10 kb down to 200 bp, with a maximum around 10 kb. If your single stranded carrier DNA consists mainly of fragments < 5 kb, we recommend that you prepare new single stranded carrier DNA, taking care not to shear the fragments during the preparation. High-quality, large fragment single stranded carrier DNA is included in the DS Yeast Transformation kit (P01003) and is also available separately from Dualsystems Biotech (P06001).
- Overgrown yeast culture. Always use an early log phase ($OD_{546} > 0.8$) yeast culture for the transformation reaction. Late log phase yeast cultures tend to have decreased transformation efficiencies.
- Amount of plasmid in the transformation reaction. Do not use less than 500 ng or more than 3 µg DNA per transformation reaction, otherwise the transformation efficiency may be severely decreased.
- PEG solution. Ensure that the PEG solution has been carefully prepared to yield a final concentration of 50 %. Follow instructions provided in the Appendix or use the PEG solution provided with the DS Yeast transformation kit (P01003).

Available accessory yeast tools

- P01003 DS Yeast Transformation kit
 P06001 Single-stranded carrier DNA

Protocol 3 Small scale expression

The small scale expression protocol is used to test five independent clones for expression of your recombinant protein. Five small scale expression cultures are set up and grown to an OD₅₄₆ of 0.8. Samples are taken at several time points, total protein extracts are prepared and your recombinant protein is detected by Western blotting with either an antibody against the HA tag or a specific antibody against your recombinant protein.

Protocol

1. Prepare five YPD (60 µg/ml G418) starter cultures by adding 5 ml of YPD (60 µg/ml G418) each to five 50 ml Falcon tubes.

Note

G418 can be added to liquid YPD medium in concentrations ranging from 60 to 200 µg/ml. The optimal concentration of G418 depends on your protein of interest. We recommend to start with 60 µg/ml G418 and to subsequently test higher G418 concentrations if desired.

2. Inoculate each tube with a single clone from your master plate.
3. Incubate the starter cultures for 6-8 hours at 30°C with shaking (200-250 rpm).
4. Inoculate the entire starter culture into 20 ml fresh YPD supplemented with the appropriate amount of G418.
5. Incubate overnight at 30°C with shaking (200-250 rpm).
6. Measure the OD₅₄₆ of each overnight culture, it should be around 1.0.

Note

If the OD₅₄₆ is significantly lower than 1.0 discard the cultures and start over again.

7. Dilute each overnight culture into 30 ml fresh YPD supplemented with the appropriate amount of G418 in a shaker flask to yield an OD₅₄₆ of 0.2.
8. Incubate the expression cultures at 30°C with shaking (200-250 rpm) until they reach an OD₅₄₆ of 0.8. This should take 3-5 hours, depending on the growth rate of the transformants.
9. Once the cultures have reached an OD₅₄₆ of 0.8 remove a 5 ml sample from each expression culture, pellet the cells by centrifugation at 4000 g for 5 minutes, discard the supernatant and quick-freeze the pellets in liquid nitrogen. Store at -80°C until further use. This is the t₀ sample.
10. Continue growth of the expression cultures for another 12-15 hours.
11. Remove another 5 ml sample from each expression culture, pellet the cells by centrifugation at 4000 g for 5 minutes, discard the supernatant and quick-freeze the pellets in liquid nitrogen. Store at -80°C until further use. This is the t₁ sample.
12. Continue growth of the expression culture for another 8-10 hours.
13. Remove another 5 ml sample from each expression culture, pellet the cells by centrifugation at 4000 g for 5 minutes, discard the supernatant and quick-freeze the pellets in liquid nitrogen. Store at -80°C until further use. This is the t₂ sample.
14. Remove all collected samples from the -80°C freezer and thaw on ice. In the meantime, prepare complete XT-buffer by mixing 7 ml of the supplied XT-buffer with the appropriate amount of protease inhibitors (we recommend

the use of protease inhibitor cocktails, such as Complete Protease Inhibitor Cocktail Tablets, Roche Applied Science, Cat.-No. 1 697 498). Always keep the complete XT-buffer on ice.

15. Add 300 μ l complete XT-buffer to each sample.
16. Set up the appropriate number of eppendorf tubes on ice.
17. Add 100 μ l of glass beads to each eppendorf tube.
18. Resuspend the pellets in the complete XT-buffer by gentle pipetting and transfer each resuspended sample to the eppendorf tube.
19. Vortex the tubes 5 times for 45 seconds each. Place the tubes on ice for 1 minute in-between vortexing steps
20. Centrifuge the samples at 14'000 g for 20 minutes in a cooled benchtop centrifuge to remove unbroken cells.
21. Transfer 200 μ l supernatant to fresh eppendorf tubes on ice.
22. Add 100 μ l SDS sample buffer to each tube.
23. Incubate at 37°C for 15 minutes
24. Centrifuge at 14'000 g for 3 minutes and store the extracts at -20°C until further use.
25. Load 10-15 μ l of each extract onto an appropriate SDS-PAGE.
26. Run the SDS-PAGE, transfer the proteins to a nitrocellulose membrane by Western Blotting and detect the recombinant protein using either an antibody against the HA tag or another antibody that is appropriate for detecting your recombinant protein.

Note

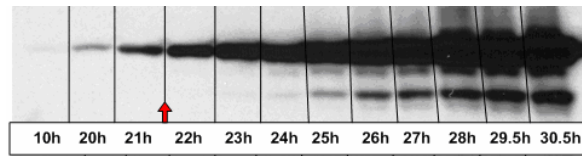
We recommend the HA.11 mouse monoclonal antibody, Covance Research Products, Cat.-No. MMS-101P.

Results



Expression of your recombinant protein should be detectable in samples t_1 and t_2 , whereas only trace amounts of your recombinant protein should be detectable in sample t_0 .

The figure below shows expression β -galactosidase from pKS1-ST. Samples were taken at the indicated time points and β -galactosidase was detected using the mouse monoclonal antibody HA.11 against the HA tag. The red arrow indicates complete depletion of glucose from the medium.



Selecting the optimal growth time

Compare the relative expression levels of your protein in samples t_1 (20 hours of growth) and t_2 (30 hours of growth). If both samples display approximately similar levels of protein or if sample t_2 displays significantly more protein than sample t_1 we recommend to grow the large scale expression culture (Protocol 4) for 30 hours. If sample t_2 displays traces of protein degradation (i.e. additional bands are visible below the expected molecular weight of your recombinant protein) or if sample t_2 displays reduced levels of your protein as compared to sample t_1 we recommend to grow the large scale expression culture only for 20 hours.

Selecting the clone with the highest expression level

Select the clone which displays the highest expression levels of recombinant protein and use this clone for further work. We recommend to prepare glycerol stocks and to restreak the master plates every two weeks.

Protocol 4 Large scale expression

To obtain sufficient amounts of recombinant protein for purification and downstream processing, the clone displaying the highest expression level in Protocol 3 is selected and used to inoculate a large scale expression culture.

Protocol

1. Prepare a starter culture by inoculating a single colony of the selected clone into YPD supplemented with the appropriate amount of G418.
2. Incubate the starter culture for 6-8 hours at 30°C with shaking (200-250 rpm).
3. Inoculate the entire starter culture into 20 ml fresh YPD supplemented with the appropriate amount of G418.
4. Incubate overnight at 30°C with shaking (200-250 rpm).
5. Measure the OD₅₄₆ of the overnight culture.
6. Dilute the overnight culture into 2 liters of YPD supplemented with the appropriate amount of G418 to yield a starting OD₅₄₆ of 0.2.

Note

Always make sure that your expression culture is well aerated. We recommend to set up 4 x 500 ml cultures in 1 liter shake flasks.

7. Grow the large scale expression culture at 30°C with shaking (200-250 rpm) for 20-30 hours (see Protocol 3 on how to determine the optimal growth time).
8. Weigh the empty centrifugation beakers (needed in step 8 to determine the pellet wet weight).
9. Centrifuge the culture at 4000 g for 20 minutes and discard the supernatant.
10. Weigh the beakers again and calculate the pellet wet weight (for 1 liter of culture at an OD₅₄₆ of 1.0, the pellet wet weight should be 5-6 grams).
11. Add 1-2 ml complete XT-buffer per gram pellet wet weight (e.g. if your pellet wet weight is 10 grams, add 10-20 ml complete XT-buffer to this pellet).
12. Resuspend the pellet by gentle pipetting and transfer to 50 ml Falcon tubes.

Note

To ensure good mixing during the vortexing step, do not add more than 25 ml of extract to each Falcon tube.

13. Add 300-600 µl glass beads per gram pellet wet weight (e.g. if your pellet wet weight is 10 grams, add 3-6 ml glass beads).
14. sVortex the tubes 5 times for 45 seconds each. Place the tubes on ice for 1 minute in-between vortexing steps
15. Centrifuge the samples at 4'000 g for 30 minutes in a cooled centrifuge to remove unbroken cells.
16. Either flash freeze the cleared extracts in liquid nitrogen or proceed directly to purification and downstream processing.

Note

Please visit www.iba-go.com for purification protocols using the Strep tag.

4 References

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5 Appendix

Reagents for yeast transformation

50 % PEG	
Reagent	For 100 ml
PEG 4000	50 g
water	80 ml

Stir until completely dissolved and adjust total volume to 100 ml with water. Sterile filter through a 0.22 μm pore filter. Aliquot into 50 ml Falcon tubes, cap tightly and seal with parafilm. It is crucial to avoid evaporation, as the concentration of PEG is a very critical parameter to achieve good transformation efficiencies.

10 x TE pH 7.5	
Reagent	For 1 liter
Tris-Cl pH 7.5	100 ml 1 M stock
EDTA pH 8.0	20 ml 0.5 M stock
water	to 1 liter

Sterile filter through a 0.22 μm pore filter and store at room temperature.

1 M LiOAc	
Reagent	For 100 ml
LiOAc x 2 H ₂ O	10.2 g
water	to 100 ml

Sterile filter through a 0.22 μm pore filter. Aliquot into 50 ml Falcon tubes, cap tightly and seal with parafilm.

Single-stranded carrier DNA from salmon sperm

1. Sterilize a 250 ml glass beaker and a stirring bar by autoclaving
2. Weigh 200 mg salmon sperm DNA type III sodium salt (Sigma D1636) into the sterile glass beaker containing the stirring bar
3. Add 100 ml sterile water
4. Dissolve the large chunks of DNA by drawing up and down a few times with a 25 ml sterile plastic pipette
5. Stir for 2-3 hours to completely dissolve the DNA. If the DNA does not dissolve, continue stirring overnight at 4 °C
6. Aliquot into sterile eppendorf tubes (1 ml per tube)
7. Boil in a water bath or heating block for 5 minutes

8. Immediately chill in an ice/water bath
9. Store at -20°C

G418 stock

Dissolve 1 g of active G418 in 5 ml water (final concentration 200 mg/ml). Filter through a 0.2 µm filter and store at -20°C. Avoid multiple freeze / thaw cycles.

Note

The amount of active G418 per gram of total material should be stated on the manufacturers datasheet.

YPD medium

YPD medium (liquid)		
Supplement	Amount / liter	Final concentration
Bacto yeast extract	10 g	1 %
Bacto peptone	20 g	2 %
Glucose monohydrate	20 g	2 %
water	to 1 liter	

Autoclave for 15 min at 121°C

YPD plates		
Supplement	Amount / liter	Final concentration
Bacto yeast extract	10 g	1 %
Bacto peptone	20 g	2 %
Glucose monohydrate	20 g	2 %
Bacto agar	20 g	2 %
water	to 1 liter	

Add a stirring bar and autoclave for 15 min at 121°C

YPD medium with G418

Liquid YPD medium: add G418 stock to the appropriate dilution directly before use. Store YPD/G418 medium at 4°C.

YPD plates: after autoclaving, cool medium to 50°C in a water bath. Add G418 stock to the appropriate dilution and pour plates. Do not store YPD/G418 plates for more than 4 weeks.

SDS sample buffer

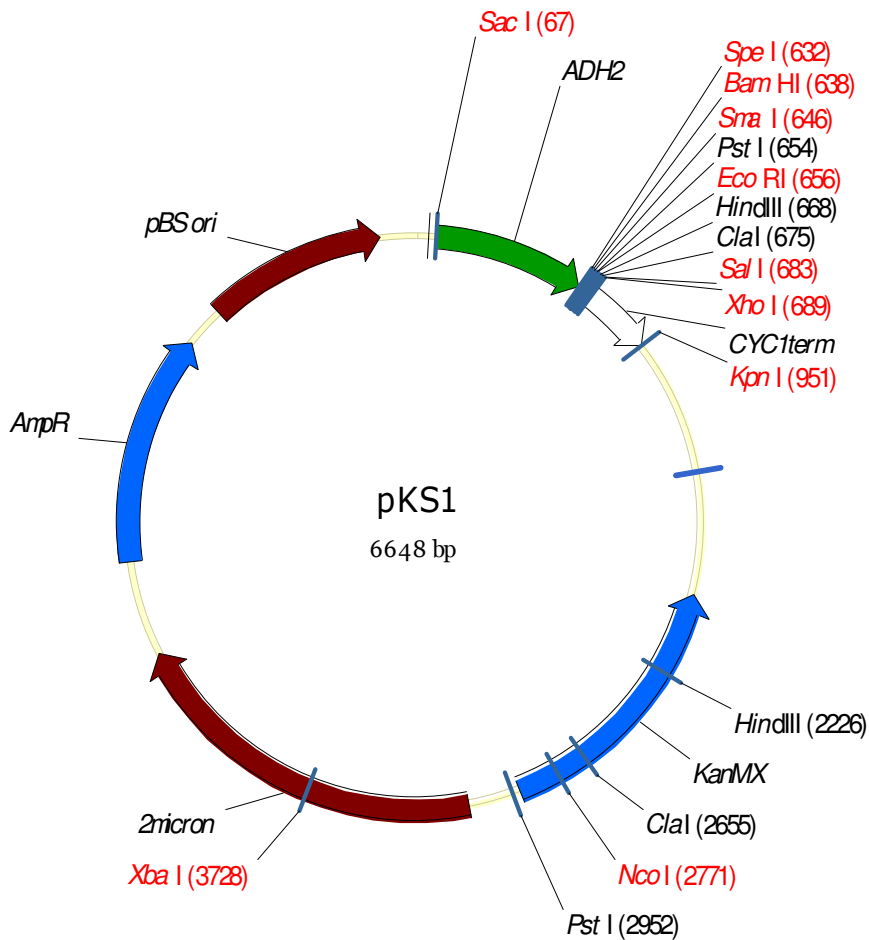
SDS sample buffer		
Reagent	Final concentration	For 50 ml
Tris-Cl pH 6.8	25 mM	1.25 ml 1 M
Urea	9 M	27 g
EDTA	1 mM	0.1 ml 0.5 M
SDS	1 %	2.5 ml 20 % stock
β -mercaptoethanol	0.7 M	2 ml
Glycerol	10 %	5 ml
water	-	to 50 ml

6 Vector information

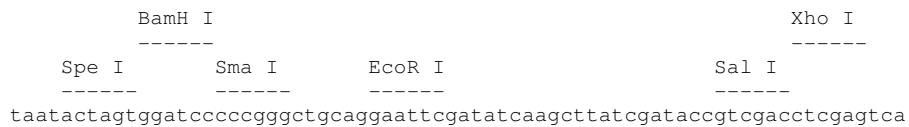
Product number	Plasmid	Promoter	Purification tag	Epitope tag	Insert	Yeast marker	E. coli marker	Origin (yeast)	Origin (E. coli)
P03305	pKS-1	ADH2	none	none	none	KanMX	AmpR	2micron, 20-100 copies/cell	pBluescript
P03306	pKS1-ST	ADH2	Strep tag	HA tag	none	KanMX	AmpR	2micron, 20-100 copies/cell	pBluescript
P03307	pKS1-ST-Gal	ADH2	Strep tag	HA tag	lacZ	KanMX	AmpR	2micron, 20-100 copies/cell	pBluescript
P03308	pKS2-ST*	ADH2	SUC2 leader and Strep tag	HA tag	None	KanMX	AmpR	2micron, 20-100 copies/cell	pBluescript

* not included in the KickStart kit

pKS1 expression vector (P03305)



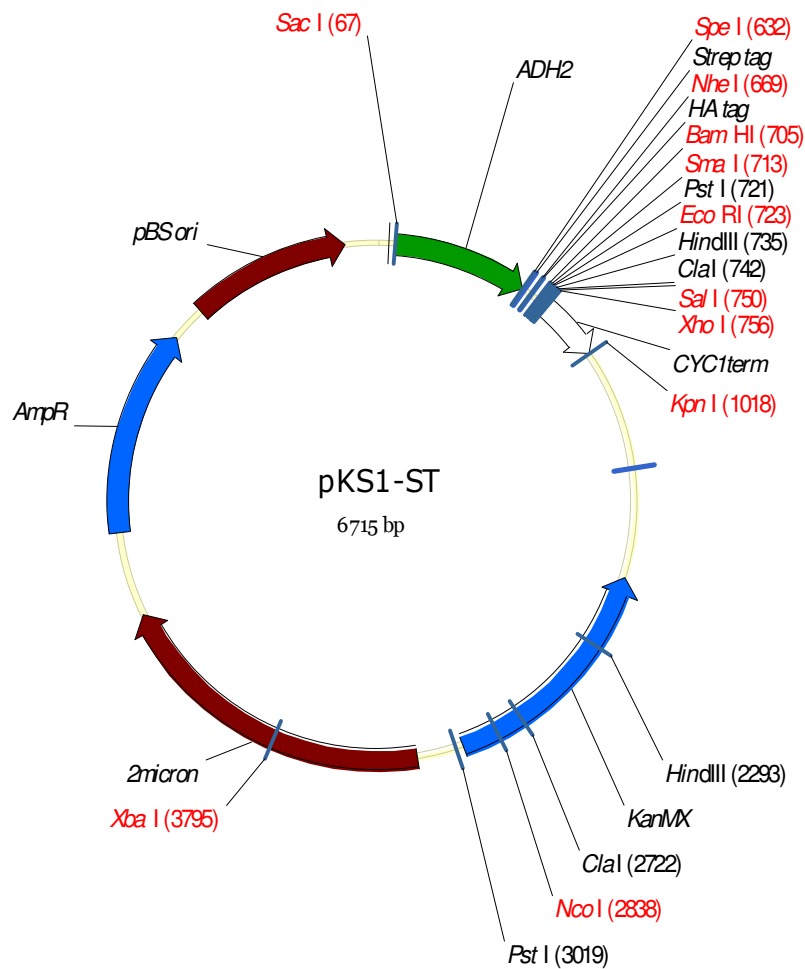
Multiple cloning site



Vector features

Position	Feature	Position	Feature
Start: 68 End: 629	ADH2 promoter	Start: 3119 End: 4486	2micron replication origin (yeast)
Start: 631 End: 694	Multiple cloning site	Start: 4842 End: 5702	AmpR resistance gene
Start: 689 End: 950	CYC1 terminator	Start: 5850 End: 6517	pBS replication origin (<i>E. coli</i>)
Start: 1919 End: 2922	KanMX cassette		

pKS1-ST Strep-tag expression vector (P03306)



Multiple cloning site

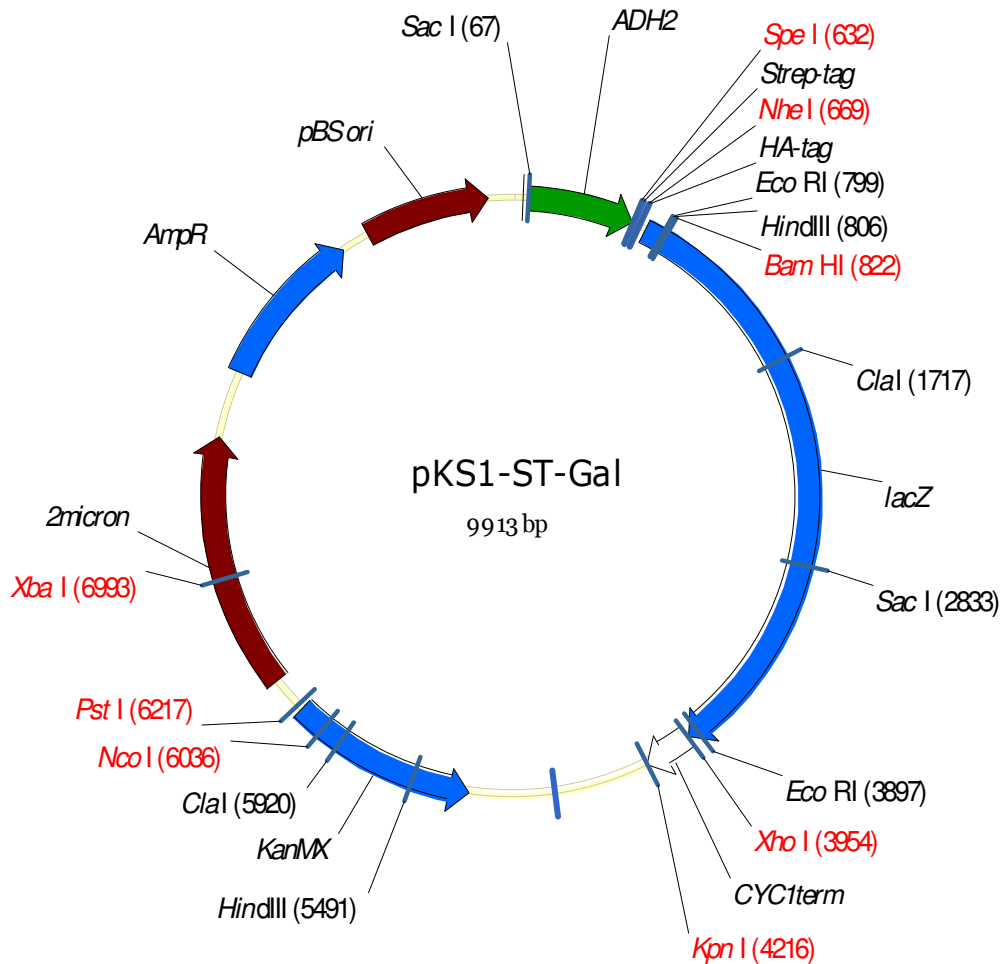
```

                BamH I   Sma I           EcoR I           Sal I   Xho I
                -----
cca gat tac gct gga tcc ccc ggg ctg cag gaa ttc gat atc aag ctt atc gat acc gtc gac ctc gag
  P   D   Y   A   G   S   P   G   L   Q   E   F   D   I   K   L   I   D   T   V   D   L   E
----->
Strep/HA-tag
    
```

Vector features

Position	Feature	Position	Feature
Start: 68 End: 630	ADH2 promoter	Start: 1986 End: 2989	KanMX cassette
Start: 641 End: 667	Strep-tag	Start: 3186 End: 4553	2micron replication origin (yeast)
Start: 674 End: 703	HA tag	Start: 4909 End: 5769	AmpR resistance gene
Start: 704 End: 761	Multiple cloning site	Start: 5917 End: 6584	pBS replication origin (<i>E. coli</i>)
Start: 756 End: 1017	CYC1 terminator		

pKS1-ST-Gal control vector (P03307)



Vector features

Position	Feature	Position	Feature
Start: 68 End: 630	ADH2 promoter	Start: 5184 End: 6187	KanMX cassette
Start: 641 End: 667	Strep tag	Start: 6384 End: 7751	2micron replication origin (yeast)
Start: 674 End: 703	HA tag	Start: 8107 End: 8967	AmpR resistance gene
Start: 710 End: 3952	lacZ (<i>E. coli</i> β -galactosidase)	Start: 9115 End: 9782	pBS replication origin (<i>E. coli</i>)
Start: 3954 End: 4215	CYC1 terminator		

7 Contact and support

If you have troubles at any point or feel that there is an error in one of the protocols, please take a look at the support section of our homepage www.dualsystems.com. If you don't find an answer to your question there, you may contact us at support@dualsystems.com and we will try to answer your questions as quickly as possible.

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